Institution: University College London		
Unit of Assessment: 9 - Physics		
Title of case study: Guiding nanoscopic probe design and developing methods to benchmark AFM performance using double helix DNA imaging		
Period when the underpinning research was undertaken: 2010-2019		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Bart Hoogenboom Alice Pyne	Professor of biophysics Research fellow in biophysics	2007 – Present 2015 – 2019
Period when the claimed impact occurred: 2010 – Present		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact (indicative maximum 100 words)

Prof. Hoogenboom's research on DNA imaging at UCL has provided Bruker, the global market leader in this area, with new methods and a benchmark to test and improve the performance of their atomic force microscopic (AFM) instruments and probes. This has resulted in increased expertise now benefiting staff in a global company; in benchmarking a new microscopy model that is now the most successful of Bruker atomic force microscopes. This research has led to the development of new probes targeted at delivering the highest resolution images in PeakForce Tapping mode that are now part of a more lucrative range of the AFM probes sold by Bruker and contributed to their USD2,100,000,000 revenue in 2019.

2. Underpinning research (indicative maximum 500 words)

Since its invention in the 1980s, atomic force microscopy (AFM) has been widely adopted to characterise surfaces at the nanoscale in contexts ranging from blue-sky academic research to, quality control in the semiconductor industry and the characterisation of shape and size of pharmaceutical compounds.

Since its establishment at UCL in 2007, Prof. Bart Hoogenboom's lab has pioneered technological advances in AFM instrumentation and made important contributions to developing methods and protocols that enhance and benchmark AFM performance, as detailed below.

AFM essentially operates like a visually impaired person reading Braille, raster-scanning a miniature "finger" – denoted as AFM probe or cantilever – over a surface at sub-nanometre precision. For optimum performance of AFM, some key elements are the design of these probes; the sensor by which any forces on these probes are detected; and the way these probes are actuated. Moreover, given the enormous diversity in AFM designs and modes of operation, it is important to have well-established benchmarks for AFM resolution.

Following his previous postdoctoral research (2002-2007) at the University of Basel, Switzerland, Professor Hoogenboom designed and developed at UCL (2007-2012) a new AFM instrument that sensed the force on an AFM probe by detecting its deflection via optical interferometry. It used laser-driven actuation to oscillate the probe in the vicinity of the sample surface and measure the proximity of the sample surface via changes in the resonance frequency of the probe. When operated under optimal conditions, Professor Hoogenboom and his team were the first to demonstrate that AFM can resolve the two strands of the DNA double helix (**R1**). Now that DNA is a widely available and easily prepared sample, the UCL-led development set a new and now generally adopted benchmark for AFM resolution. Professor Hoogenboom's lab also demonstrated that AFM performance critically depends on the quality of the actuation and detection of the AFM probe, beyond the standard of actuation and detection that was available in commercial instruments at that time (**R1**).

Having achieved such AFM performance on an instrument modified in-house, the UCL team set out to establish if similar performance could be achieved on instruments that were more widely available (2010-2014). This started with a beta-version of a commercial instrument, made available by AFM manufacturer Bruker. By screening various AFM probes and imaging parameters, Professor Hoogenboom's team established protocols that could be adopted by other AFM users to obtain DNA double helix resolution (**R2**). The research showed that AFM data on DNA provides quantitatively accurate information on DNA double helix structure, if the analysis also considers the dimensions of the AFM probe and the forces involved in the imaging process (**R2)**, therefore, demonstrating that such high resolution can also be obtained on commercially available instruments.

In follow-up research, these methods were also used by Professor Hoogenboom's lab to study the formation of DNA quadruplexes (**R3**), the binding of DNA repair proteins that are targets for anti-cancer drugs (**R4**), and the formation of DNA triplexes.

Prof. Hoogenboom was awarded the 2017 Medal for Scanning Probe Microscopy in part for his AFM work on DNA double helix imaging.

3. References to the research (indicative maximum of six references)

R1. Leung C, Bestembayeva A, Thorogate R, Stinson J, **Pyne A**, Marcovich C, Yang J, Drechsler U, Despont M, Jankowski T, Tschope M, **Hoogenboom BW** (2012), Atomic force microscopy with nanoscale cantilevers resolves different structural conformations of the DNA double helix, *Nano Letters* 12 : 3846–3850, https://doi.org/10.1021/nl301857p.
[80 citations, google scholar]
This research was highlighted, among other places, in Nature Methods 9 (2012): 778–779.

R2. **Pyne A**, Thompson R, Leung C, Roy D, **Hoogenboom BW**, (2014) Single-molecule reconstruction of oligonucleotide secondary structure by atomic force microscopy, *Small* 10: 3257–3261 doi: https://doi.org/10.1002/smll.201400265.
[85 citations, google scholar]

R3. Klejevskaja B, **Pyne A**, Reynolds M, Shivalingam A, Thorogate R, **Hoogenboom BW**, Ying L, Vilar R (2016) Studies of G-quadruplexes formed within self-assembled DNA mini-circles, *Chemical Communications* 52:12454–12457, doi:http://doi.org/10.1039/c6cc07110d [11 citations, google scholar]

R4. Akpinar B, Haynes PJ, Bell NAW, Brunner KW, **Pyne A**, **Hoogenboom BW** (2019) PEGylated surfaces for the study of DNA–protein interactions by atomic force microscopy, *Nanoscale* 11: 20072–20080, https://doi.org/10.1039/C9NR07104K [3 citations, google scholar]

4. Details of the impact (indicative maximum 750 words)

Atomic force microscopes have been commercially available since the early 1990s. Since then, several companies have built and sold AFM instruments, including Bruker (US), Agilent (US), JEOL (Japan), Park Systems (South Korea), Oxford Instruments (UK), Nanosurf (Switzerland), Hitachi (Japan), Horiba (Japan), WITec (Germany), NT-MDT (Russia), NanoMagnetics Instruments (UK) and Nanonics Imaging (Israel). Such instruments are now routinely used for surface analysis in academic and industrial research. In 2019, the AFM market was worth USD441,000,000 and is projected to reach USD586,000,000 by 2024 (**S1**). By various acquisitions and further developing AFM, Bruker has become the market leader in this field. Its Bruker Nano arm of the business, which Professor Hoogenboom has been collaborating with for AFM, accounted for approximately 30% (USD632,700,000) of the company's USD2,100,000,000 revenue in 2019 (**S2**).

Key parts of the commercial success of Bruker's AFM instrumentation have been the continuous technology development towards achieving high-resolution imaging with decreasing amounts of user input, and of AFM probes that are of more consistently high quality. These developments have been inspired and supported by research at UCL and led to improvements in instrumentation; protocols for benchmarking the quality of AFM instruments as demonstrated by double helix DNA imaging; and guiding the development of a new AFM probe.

Contributions to improving Bruker's AFM instrumentation

The relationship between UCL and Bruker emerged in 2012 stemming from AFM instrument development in Professor Hoogenboom's lab (**R1**). This led to the establishment of a joint development agreement between UCL and Bruker, with the overall aim to improve Bruker instrumentation to realise the high resolution achieved in published work from UCL which demonstrated DNA double-helix imaging. The collaboration with Bruker involved improving a beta-version of a Bruker AFM microscope. The instrument was **modified to reduce the noise in the optical detection of the AFM probe and it included a new proprietary method of cantilever actuation and imaging mode,** named "PeakForce Tapping".

Guided by investigations at UCL and at its headquarters by UCL researchers, Bruker used data from these experiments to improve its equipment, as well as the robust and reproducible characterisation of DNA at double helix resolution on Bruker instruments as detailed in their published paper (**R2**). The resulting methods have **continued to be used at Bruker as a standard protocol to optimise imaging parameters**.

Developing benchmarking methods to demonstrate high quality instrumentation and improving promotional material

To guide and demonstrate the success of improvements to instrumentation and/or methods, well-defined benchmarks are needed. An ideal benchmark is representative of a large class of AFM samples (actual and potential) that are inexpensive, widely available, easy to prepare for AFM; and sets a high bar for instrument performance. The Hoogenboom group's research on imaging DNA and resolving the major and minor grooves of the double helix has become the benchmark in the Bio AFM market; as the criterion of double-helix resolution puts high demands on the way in which AFM probes are actuated and detected and on the quality of the probe. The high-quality DNA double-helix imaging provided an accessible and easily recognisable benchmark for Bruker's PeakForce Tapping mode against its competitors and strengthened Bruker's position with respect to competitors. The Director of AFM Product Management and Applications Development at Bruker Nano Surfaces stated: "As a result of the improved sample preparation protocol and the quality DNA double helix images Dr Pyne had captured, we were then **able to use these images, benchmark PeakForce Tapping Mode against peer methods and provide proof of performance in some of our tender responses"** (**S3**).

Furthermore, Bruker used the research and protocols pioneered by the Hoogenboom group as input for an application note 142: *Imaging of the DNA Double Helix with PeakForce Tapping Mode Atomic Force Microscopy* (**S4**) and promotional material as exemplified by the cover illustration of the 2013 edition of the journal *Microscopy & Analysis* (**S5**). The application note and promotional materials have been included in presentations by Bruker's representatives, as they served to demonstrate the performance of Bruker instrumentation to existing and prospective customers; therefore widening the use and customer base of the company.

Improving training and development for company employees and customers

In the wake of UCL's publication on DNA imaging (**R1**), Dr Pyne from UCL travelled to Bruker Nano's R&D Headquarters in Santa Barbara, California in 2015 where she delivered hands-on training, based on the methods for high-resolution DNA imaging developed at UCL to ten of their approximately 40 Application Scientists representing each of Bruker's sales regions (NA, EU, APAC, Japan). A Senior Sales Engineer at Bruker UK confirmed learning from the workshop has continued to be used "These Applications Scientists have all used this **training as part of their customer systems demonstrations and several have provided the same training to other Apps Scientists** in their respective regions." (**S3**)

Dr Pyne also provided a written manual to Bruker's application scientists, enabling them to demonstrate Bruker instrument performance to customers worldwide. Bruker's Director of

Product Management and Applications Development commented: “The manual on the scanning parameters for single molecule DNA, co-authored by [UCL scientist] Dr. Alice Pyne and Dr. Andrea Slade (Bruker), has **allowed this invaluable knowledge to be passed on within the company (including to Bruker staff in EMEA, USA, Japan and Asia Pacific regions) and to customers alike**” (**S3**). This manual has provided “reference material to assist them [customers] in high-resolution imaging studies of their own samples”, which has facilitated the delivery of Bruker’s “larger goal […of] enabling our customers to do more new and exciting research” (**S3)**.

Impact on development and sales of AFM probes

In addition to AFM instrumentation, Bruker also develops and sells AFM probes to use with its own and other AFM systems. Complemented by experiments carried out by UCL scientists in London and Santa Barbara, the UCL research on DNA imaging facilitated Bruker’s development, testing, and benchmarking of new lines of AFM probes (**R1, 2**). The impact of the Hoogenboom group’s work on directing probe development is corroborated by testimony from Bruker, “the knowledge gain from the collaboration with Dr Pyne and Professor Hoogenboom has **led to focused investment**” and that “During our collaboration, Alice [Pyne] was a member of the Bruker Team who, together with Bruker Probes Engineers, Dev Apps Scientists, and Sales Apps Scientists, **worked on the development of specialized high-resolution imaging AFM probes**” (**S3**). The most tangible result of this has been the development of what is now Bruker’s PeakForce-HIRS-F-A/B product line (**S6**). These probes were first launched in 2015 as a direct result of Dr. Pyne’s benchmarking and prototype evaluation work on DNA and generated a new source of revenue for the company. The probes were the first to specifically enable high-resolution imaging on single biomolecules as they were designed for robust double helix imaging. Due to the high technical specification of these probes, they are sold in multiples of 10 at more than GBP50 per probe. This is more than double that of other probe models available from Bruker. The PEAKFORCE-HIRS-F-B probes are advertised as “**The world's first probe targeted at delivering high resolution on single biomolecules**” and are recommended for “…highest resolution fluid imaging of individual, isolated bio-sample such as resolving the DNA double helix - the BioAFM market's standard for demonstrating highest resolution performance” (**S6**). A Senior Sales Engineer at Bruker UK said: “Our [Bruker’s] **customers have confirmed that these probes are indeed very useful for AFM studies** in molecular biology and we continue to offer them as part of our high-end probe catalogue” (**S3**). In 2017 the Bruker Nano Group reported a **USD58,400,000 increase in revenue on the previous year**. Much of this increase has been “driven by solid performance in academic and industrial materials research markets for our X-Ray, nano surfaces and nano analysis tools” (**S2**). This increase also corresponds to the introduction of PeakForce Hi-Res F-A/B series demonstrating that sales from the line have contributed to the increase in Bruker’s revenue.

5. Sources to corroborate the impact (indicative maximum of 10 references)

S1. References the of overall market for AFM instrumentation- Accessed 26/02/21- corroborates market value of AFM

S2. Snapshot of Bruker’s financial details from their annual Corp10-K report published in 2020 – Accessed 26/02/21- corroborates financial data for Bruker in 2019

S3. Testimonial letters from Bruker employees Thomas Mueller and Stephen Lewandowski to corroborate impact of UCL research on Bruker’s instrumentation R&D and training.

S4. Application note #142 “Imaging of the DNA Double Helix with PeakForce Tapping Mode Atomic Force Microscopy”- evidences methods to achieve DNA Double Helix imaging in Tapping Mode.

S5. Microscopy & Analysis cover image + accompanying text on p. 3, January 2013 (PDF of journal pages is also available)

S6. Overview of some of Bruker’s PeakForce HIRS product line- corroborates development of Bruker’s high resolution probes